ASSESSMENT OF CONTEMPORARY ROOT CANAL FILE SYSTEMS: SIZES, SHAPES AND THE EFFECTIVENESS OF 31-GAUGE IRRIGATION NEEDLE IN INTRACANAL BACTERIAL REDUCTION

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A thesis submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the School of Dentistry (Endodontics).

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ABSTRACT

Melita Islambasic: Assessment of Contemporary Root Canal File Systems: Sizes, Shapes and the Effectiveness of 31-Gauge Irrigation Needle In Intracanal Bacterial Reduction
(Under the direction of Peter Z. Tawil)

In Part I, the effects of irrigant delivered via a 31-gauge irrigation needle to E. *faecalis* infected teeth instrumented to different apical sizes with six different nickel titanium (NiTi) rotary instruments were examined in an in-vitro model using 165 single canaled extracted teeth. In Part II, the accuracy and deformation of NiTi rotary files before and after use were examined via SEM imaging and ImageJ software. Statistical analysis was done using r-ANOVA and Tukey's test for Part I, and proportional odds model and Mantel-Haenszel row mean score for Part II. Smaller preparation sizes showed higher likelihood of having a larger bacterial count compared to larger preparation sizes. Most files' nominal diameter and taper did not conform to their advertised size showing a lack of standardization between manufacturers. Pilot tip lengths vary greatly between different rotary files.

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LIST OF ABBREVIATIONS

ANSI/ADA	American National Standards Institute/American Dental Association
EDTA	Ethylene Diamine Tetraacetic Acid
ISO	International Standards Organization
NaOCl	Sodium Hypochlorite
NiTi	Nickel-Titanium
NRG	New Recommended Guideline
TPT	True Pilot Tip
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth

CHAPTER 1 REVIEW OF LITERATURE

INTRODUCTION

The etiology of pulpal and periapical disease is the bacteria and their by-products (1, 2). The goal of endodontic therapy is to eliminate bacteria, pulpal tissue and debris from the root canal system using mechanical instrumentation and chemical disinfection while maintaining aseptic techniques (3). This is followed by a root canal filling that should seal the root canal system thus preventing future influx of bacteria from the coronal and/or apical aspect, as well as to entomb any residual microorganisms that might remain (4). A number of protocols have been suggested to accomplish this task: some support larger apical size (5), while others prefer greater continuous taper (6).

The debate over what is the appropriate apical preparation size and taper has been going on for the past couple of decades. Even though numerous publications are available on the subject of root canal preparation, there is still no conclusive scientific evidence as to what is the best instrumentation and technique protocol (7). Partially, this could be attributed to the fact that mechanical cleaning and shaping of the root canal is only 65% effective in touching all of the canal walls (8), thus leaving a lot of debris and untouched surfaces which may harbor the pathogens we are trying to eliminate. It is known that the irrigant plays a major role in disinfecting the root canal space (9), thus one of the purposes of the mechanical preparation is to allow the irrigant to reach the most apical aspect of the root canal system. Multiple articles have been published suggesting a necessary size of the root canal enlargement in order to disinfect it. Card *et al*.(5) showed that by enlarging the mandibular cuspids and bicuspids to a size 80 (by using LightSpeed rotary), negative cultures were obtained from the roots prior to obturation when using a 28-gauge needle. Peters *et al*. (10) found that it may not be necessary to obtain a negative culture prior to obturation in order to have a favorable endodontic outcome during a five year follow up study. Multiple articles have reported on numerous pathogens responsible for the development of apical periodontitis (11, 12). In our quest to eliminate the pathogens in the apical deltas and ramifications, increasing the canal preparation size allows the irrigant to reach the most apical aspects of the root, but it also weakens the root and makes it more susceptible to dentinal defects that can lead to cracks, fractures and unfavorable long term prognosis (13-15).

Khademi *et al.* (16) showed that when using a 27-gauge irrigation needle, the minimal apical preparation size of 30.06 was required for the irrigant to reach the apical third of the root. It is important to be able to effectively clean, shape and disinfect the apical third of the root due to the numerous deltas and ramifications that are commonly encountered in this region of the root canal systems, especially of premolars and molars (17). A number of papers have been published evaluating the irrigant penetration in the root utilizing 23, 27, 28 and 30-gauge needles (5, 9, 16, 18-20). When talking about different gauge sizes, it is important to keep in mind that a lower number gauge corresponds to a broader needle diameter, and a higher number gauge needle corresponds to a narrower or smaller needle diameter (for example, 23-gauge needle (lower number gauge) has a diameter of 0.637mm, and a 31-gauge needle (higher number gauge) has a diameter of 0.2605mm). A publication that governs tubing dimensions is ISO 9626 (21).

Albrecht *et al*. (20) reported that when evaluating the distance between the 28-gauge irrigation needle (designated metric size 0.36mm, with the outside diameter range of 0.349-0.370mm) and the apices of anterior and premolar teeth that have been instrumented to a size 40 with a .04 taper, the irrigation needle was approximately 2.25mm short of the working length when inserted in the canal without binding. The suggested explanations were that this may be due to the parallelism of the canal walls in a .04 taper preparation, as well as to a slight but sufficient amount of canal curvature which might have impeded the full needle penetration. It has been shown that delivering the irrigant with a higher number gauge needle which has a narrow diameter is more desirable (18, 22, 23) because it allows for more apical placement of the irrigation needle. It has also been shown that the reduction of the microbes is directly correlated to the total volume of the irrigant used (24). However, we need to be aware that with the higher number gauge needle, it will take longer to extrude the same amount of volume when compared to the lower number gauge needle (23). This is due to the flow resistance in needles with a higher number gauge.

With the intracanal instrument size of 30, if there are no obstacles, a 31-gauge needle should be able to reach within a millimeter from the apex in a canal that has been prepared to a minimal size of 25.04 or 25.06. Many publications evaluated and compared several different rotary instruments and irrigation protocol effectiveness in bacterial reduction (5, 16, 24-27). With our present study, we wanted to add to this body of knowledge by evaluating the amount of bacterial reduction using smaller apical sizes of rotary preparation in conjunction with higher numbered (31-gauge, 0.2605mm) irrigation needle with dual side port vents.

Recent improvements and innovations in the manufacturing process of the nickel titanium (NiTi) rotary instrumentation have been able to provide greater flexibility of the files, thus increasing their torsional strength and ability to negotiate a challenging root canal system with lesser chance of separation (28). If we add to this a narrow diameter of a 31-gauge irrigation needle, we may postulate that it may be possible to place the irrigation needle deeper into the root while maintaining conservative root canal preparation. Historically, the advocates of the "standardized technique" or apical stop preparation (29) suggested that the canal should be prepared to at least three sizes larger than the first file that binds at the working length. Some of the downsides of this technique include inaccurate determination of working length if the canal is not enlarged coronally prior to making working length determination (31), unnecessary enlargement of small canals, apical perforation and transportation. On the other hand, the advocates of the tapered apical control zone technique (32) want to maintain the anatomy and the position of the apical foramen by leaving the apical preparation size as small as possible while creating a more tapered canal preparation (30). This creates a "linear resistance form" (6) which minimizes the potential of gutta percha extrusion, as well as canal transportation and perforation.

Regardless of which instrumentation technique is used in the endodontic procedure, what may be more important is the knowledge and awareness of the file sizes that are being introduced into the root canal system. Thanks to the efforts of Green (33), Ingle (29, 34) and others who in the mid-1950's proposed standardization of endodontic files, we have the privilege of combining different rotary systems while achieving and/or maintaining a desired size. Manufacturers of endodontic files rely on the specifications set by the International Standards Organization (ISO 3630-1) (35) and American National Standards Institute/American Dental Association (ANSI/ADA Spec 101) (36) to ensure their files specifications fall within the allowable tolerance for their advertised sizes. However, even with the guidelines in place, reports of files not fitting the requirements are available. Evaluation of stainless steel H- and K-files, as well as rotary NiTi files by Zinelis *et al*. (37) showed that none of the files tested had the advertised nominal diameter. Lask *et al*. (38) evaluated diameter and taper of four different brands of size 30.04 NiTi rotary files, and found that files tended to be larger than the nominal size, but concluded that the differences were minute and probably not clinically relevant. Kim *et al*. (39) evaluated dimensional standard of several NiTi rotary files, and found that the diameter values were mostly not in compliance with the advertised value.

With newer more flexible rotary file systems becoming available, we need to be cognizant of the amount of deformity that occurs with each file and its effect on the final size of root canal preparation we are trying to achieve. Nickel-titanium files have a unique property of shape memory (the ability of a deformed file to bounce back into the original shape) and superelasticity (the ability to return to original shape upon unloading before deformation occurs) (40). One of the characteristics of the newer files is their ability to undergo "pseudoelastic recoverable deformation" (41), which allows them to unwind when placed under torsional stress thus increasing their fatigue resistance. The manufacturers' state that the unwinding feature is a safety mechanism which increases the resistance of a file to separation. To our knowledge, there have been no reports on the amount of unwinding that occurs, as well as on the resulting size of the file when it unwinds. The unwinding feature implies that the file size changes as the file is stretched. When correlating this feature to a clinical situation, it would appear that the shape and size of the canal preparation may not be the same as the original size of the file used, due to its deformity during use. It is also important to consider how this change affects the depth of irrigation needle penetration, and thus the efficacy of the irrigant that is being delivered. In our present study, we evaluated the accuracy and the amount of file deformity that occurred during instrumentation. Nominal diameter, taper and pilot tip lengths were recorded for each file before and after use, and analyzed for statistical relevance.

The null hypothesis was that there will be no difference in using smaller vs. larger size rotary instruments in conjunction with a 31-gauge irrigation needle in achieving bacterial reduction, and that file sizes will not change before and after use. The alternative hypothesis was that there will be a difference in bacterial reduction between smaller and larger rotary instruments, and that the file deformation will occur.

REFERENCES

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The Effects of Surgical Exposures of Dental Pulps in Germ-Free and Conventional Laboratory Rats. Oral surgery, oral medicine, and oral pathology 1965;20:340-349.

2. Moller AJ, Fabricius L, Dahlen G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scandinavian journal of dental research 1981;89(6):475-484.

3. Peters OA, Peters CI. Cleaning and shaping of the root canal system. In: Cohen S, Pathways of the Pulp. 10th edition, St. Louis, MO: Mosby Company, 2011: 283-348.

4. Sundqvist G, Figdor D. Endodontic treatment of apical periodontitis. In: Orstavik D, Pitt Ford TR, eds. Essential Endodontology: Prevention and Treatment of Apical Periodontitis. London, UK. Blackwell Science Ltd. 1998:242-269.

5. Card SJ, Sigurdsson A, Orstavik D, Trope M. The effectiveness of increased apical enlargement in reducing intracanal bacteria. Journal of endodontics 2002;28(11):779-783.

6. Buchanan LS. Tapered shaping objectives can make your life easier! Dentistry Today 2010;29(1):112, 114-119.

7. Hulsmann M, Peters OA, Dummer PMH. Mechanical Preparation of Root Canals: Shaping Goals, Techniques and Means. Endodontic Topics 2005(10):30-76.

8. Peters OA, Schonenberger K, Laib A. Effects of four Ni-Ti preparation techniques on root canal geometry assessed by micro computed tomography. International endodontic journal 2001;34(3):221-230.

9. Siqueira JF, Jr., Rocas IN, Favieri A, Lima KC. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. Journal of endodontics 2000;26(6):331-334.

10. Peters LB, Wesselink PR. Periapical healing of endodontically treated teeth in one and two visits obturated in the presence or absence of detectable microorganisms. International endodontic journal 2002;35(8):660-667.

11. Fujii R, Saito Y, Tokura Y, Nakagawa KI, Okuda K, Ishihara K. Characterization of bacterial flora in persistent apical periodontitis lesions. Oral microbiology and immunology 2009;24(6):502-505.

12. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Critical reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists 2004;15(6):348-381.

13. Shemesh H, Bier CA, Wu MK, Tanomaru-Filho M, Wesselink PR. The effects of canal preparation and filling on the incidence of dentinal defects. International endodontic journal 2009;42(3):208-213.

14. Liu R, Kaiwar A, Shemesh H, Wesselink PR, Hou B, Wu MK. Incidence of apical root cracks and apical dentinal detachments after canal preparation with hand and rotary files at different instrumentation lengths. Journal of endodontics 2013;39(1):129-132.

15. Tawil PZ, Saraiya VM, Galicia JC, Duggan DJ. Periapical microsurgery: the effect of root dentinal defects on short- and long-term outcome. Journal of endodontics 2015;41(1):22-27.

16. Khademi A, Yazdizadeh M, Feizianfard M. Determination of the minimum instrumentation size for penetration of irrigants to the apical third of root canal systems. Journal of endodontics 2006;32(5):417-420.

17. De Deus QD. Frequency, location, and direction of the lateral, secondary, and accessory canals. Journal of endodontics 1975;1(11):361-366.

18. Guerreiro-Tanomaru JM, Loiola LE, Morgental RD, Leonardo Rde T, Tanomaru-Filho M. Efficacy of four irrigation needles in cleaning the apical third of root canals. Brazilian dental journal 2013;24(1):21-24.

19. Hockett JL, Dommisch JK, Johnson JD, Cohenca N. Antimicrobial efficacy of two irrigation techniques in tapered and nontapered canal preparations: an in vitro study. Journal of endodontics 2008;34(11):1374-1377.

20. Albrecht LJ, Baumgartner JC, Marshall JG. Evaluation of apical debris removal using various sizes and tapers of ProFile GT files. Journal of endodontics 2004;30(6):425-428.

21. International Standards Organization. Stainless steel needle tubing for the manufacture of medical devices. ISO 9626:1991/Amd.1:2001(E)

22. Abou-Rass M, Piccinino MV. The effectiveness of four clinical irrigation methods on the removal of root canal debris. Oral surgery, oral medicine, and oral pathology 1982;54(3):323-328.

23. Chow TW. Mechanical effectiveness of root canal irrigation. Journal of endodontics 1983;9(11):475-479.

24. Sedgley CM, Nagel AC, Hall D, Applegate B. Influence of irrigant needle depth in removing bioluminescent bacteria inoculated into instrumented root canals using real-time imaging in vitro. International endodontic journal 2005;38(2):97-104.

25. Baratto-Filho F, Leonardi DP, Zielak JC, Vanni JR, Sayao-Maia SM, Sousa-Neto MD. Influence of ProTaper finishing files and sodium hypochlorite on cleaning and shaping of

mandibuldar central incisors--a histological analysis. Journal of applied oral science : revista FOB 2009;17(3):229-233.

26. Coldero LG, McHugh S, MacKenzie D, Saunders WP. Reduction in intracanal bacteria during root canal preparation with and without apical enlargement. International endodontic journal 2002;35(5):437-446.

27. Falk KW, Sedgley CM. The influence of preparation size on the mechanical efficacy of root canal irrigation in vitro. Journal of endodontics 2005;31(10):742-745.

28. Shen Y, Qian W, Abtin H, Gao Y, Haapasalo M. Effect of environment on fatigue failure of controlled memory wire nickel-titanium rotary instruments. Journal of endodontics 2012;38(3):376-380.

29. Ingle JI. A standardized endodontic technique utilizing newly designed instruments and filling materials. Oral surgery, oral medicine, and oral pathology 1961;14:83-91.

30. Schilder H. Cleaning and shaping the root canal. Dental clinics of North America 1974;18(2):269-296.

31. Weine FS, Kelly RF, Lio PJ. The effect of preparation procedures on original canal shape and on apical foramen shape. Journal of endodontics 1975;1(8):255-262.

32. Serota KS, Nahmias Y, Barnett F, Brock M, Senia ES. Predictable endodontic success. The apical control zone. Dentistry today 2003;22(5):90-97.

33. Green EN. Microscopic investigation of root canal file and reamer widths. Oral surgery, oral medicine, and oral pathology 1957;10(5):532-540.

34. Ingle JI. The need for endodontic instrument standardization. Oral surgery, oral medicine, and oral pathology 1955;8(11):1211-1213.

35. International Standards Organization. Dentistry - Root-canal instruments. Part 1: General Requirements and Test Methods. ISO 3630-1:2008(E) 2008.

36. American Dental Association Council on Scientific Affairs. ANSI/ADA Specification No. 101. Root Canal Instruments: General Requirements.. 2001. Reaffirmed Oct 2010.

37. Zinelis S, Magnissalis EA, Margelos J, Lambrianidis T. Clinical relevance of standardization of endodontic files dimensions according to the ISO 3630-1 specification. Journal of endodontics 2002;28(5):367-370.

38. Lask JT, Walker MP, Kulild JC, Cunningham KP, Shull PA. Variability of the diameter and taper of size #30, 0.04 nickel-titanium rotary files. Journal of endodontics 2006;32(12):1171-1173.

39. Kim KW, Cho KM, Park SH, Choi KY, Karabucak B, Kim JW. A comparison of dimensional standard of several nickel-titanium rotary files. Restorative dentistry & endodontics 2014;39(1):7-11.

40. Thompson SA. An overview of nickel-titanium alloys used in dentistry. International endodontic journal 2000;33(4):297-310.

41. Peters OA, Gluskin AK, Weiss RA, Han JT. An in vitro assessment of the physical properties of novel Hyflex nickel-titanium rotary instruments. International endodontic journal 2012;45(11):1027-1034.

CHAPTER 2 MANUSCRIPT #1
ASSESSMENT OF CONTEMPORARY ROOT CANAL FILE SYSTEMS: SIZES, SHAPES AND THE EFFECTIVENESS OF 31-GAUGE IRRIGATION NEEDLE IN INTRACANAL BACTERIAL REDUCTION

INTRODUCTION

The etiology of pulpal and periapical disease is the bacteria and their by-products (1, 2). The goal of endodontic therapy is to eliminate bacteria, pulpal tissue and debris from the root canal system using mechanical instrumentation and chemical disinfection (3). This is followed by a root canal filling which should seal the root canal system thus preventing future influx of bacteria from the coronal and/or apical aspect, as well as to entomb any residual micro-organisms that might remain (4).

Mechanical cleaning and shaping of the root canal touches about 65% of the canal walls (5), leaving debris and untouched surfaces which may harbor pathogens we are trying to eliminate. Instrumentation without the use of an irrigant leaves approximately 70% more debris in the canal space when compared to the canals instrumented with irrigation (6). Irrigant plays a major role in disinfecting the root canal space (7) and one of the purposes of the mechanical preparation is to allow the irrigant to reach the most apical aspect of the root canal system. Multiple articles have been published suggesting a necessary size of the root canal enlargement in order to disinfect it. Card *et al*. (8) showed that enlarging the mandibular cuspids and bicuspids to a size 80 (by using LightSpeed rotary) provided negative cultures prior to obturation when using a 28-gauge needle. Peters *et al*. (9) found that it may not be necessary to obtain a negative culture prior to obturation in order to have a favorable endodontic outcome during a five year follow up study. Multiple articles have reported on numerous pathogens responsible for the development of apical periodontitis (10, 11). In our quest to eliminate or at least reduce the pathogens in the apical deltas and ramifications, increasing the canal preparation size allows the irrigant to reach the most apical aspects of the root, but it also weakens the root and makes it more susceptible to dentinal defects that can lead to cracks and fractures (12-14).

Khademi *et al.* (15) showed that when using a 27-gauge irrigation needle, the minimal apical preparation size of 30.06 was required in order for the irrigant to reach the apical third of the root and remove debris and smear layer. It is important to be able to effectively clean, shape and disinfect the apical third of the root due to the numerous deltas and ramifications that are commonly encountered in this region of the root canal systems, especially of premolars and molars (16). A number of papers have been published evaluating the irrigant penetration in the root utilizing 23, 27, 28 and 30-gauge needles (7, 8, 15, 17-19).

Delivering the irrigant with a higher number gauge needle which has a narrow diameter is more desirable (17, 20, 21) because it allows more apical placement of the irrigation needle. It has also been shown that the reduction of the microbes is directly correlated to the total volume of the irrigant used (22). Studies have been done evaluating and comparing several different rotary instruments and irrigation protocol effectiveness in bacterial reduction (8, 15, 22-25). With our present study, we wanted to add to this body of knowledge using a newer and smaller 31-gauge needle.

The purpose of this in-vitro study was to evaluate the amount of intracanal bacterial reduction after instrumentation with different sizes of NiTi rotary files and irrigation with a 31-gauge needle.

EXPERIMENTAL METHOD

Extracted teeth preparation

A total of 165 extracted single canaled teeth were used. Teeth were stored in 0.5% NaOCl. Inclusion criteria: single canaled teeth with narrow and restricted canal space (maxillary and mandibular anteriors and premolars) where a size #15 K-file met resistance during negotiation; minimal curvature (<15°); acceptable restoration. Exclusion criteria: multiple canals, fracture lines, curvature beyond 15 degrees. Teeth were radiographed buccolingually and mesiodistally to evaluate compliance with inclusion/exclusion criteria. The length of the teeth was standardized to 20mm via enameloplasty with a high speed diamond bur (SS White, Lakewood, NJ). Teeth were accessed using a carbide FG 2R bur (SS White, Lakewood, NJ), negotiated with 6-8-10 C-files (Dentsply Tulsa, Tulsa, OK) and instrumented 0.5mm beyond the apical foramen with a size #15 K-file (Dentsply Tulsa, Tulsa, OK) under a dental operating microscope (Global Surgical Co, St. Louis, MO). Tap water irrigation with NaviTip 31-gauge double sideport side-vented irrigation needle (Ultradent Products Inc., South Jordan, UT) was used during the canal negotiation. After preparation, teeth were placed in 0.5% NaOCl for 24 hrs, rinsed with sterile ionized distilled water (5 volume changes, 2 min each) and air dried. Entire root surface and the apical foramina were sealed with nail varnish to prevent bacterial leakage. Teeth were placed vertically in a hinged lid microtube storage box (Argos Tech, Elgin, IL) with Miltex replacement sponge (Integra Miltex, York, PA) on the bottom (roots were not allowed to touch the sponge) and sterilized overnight by ethylene oxide gas.

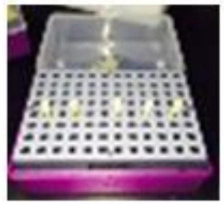


Figure 1.1. Microtube storage box with prepared teeth.

Bacterial suspension and root infection

Bacterial suspension was prepared by placing a pure isolated 24-hour clinical colonies of E. *faecalis* grown on trypticase soy agar (TSA) 5% sheep blood plates (Spectrum Scientific, Philadelphia, PA) for 24 hours into a vial containing 15ml of tryptic soy broth (TSB). After 8 hours, one drop of the suspension was transferred to a new vial with fresh TSB in order to avoid saturation. This process was repeated twice every 12 hours to allow for bacterial acclimation. Bacterial suspension was adjusted to match the turbidity of 1.5×10^8 colony-forming units (CFUs)/mL (equivalent to 0.5 McFarland standard) and diluted to 10^6 CFU. 1ml of 10^6 suspension was used for the inoculation of each experimental and positive control tooth using sterile 10-ml syringe (BD Co, Franklin Lakes, NJ) with a 31-gauge needle. The inoculum was pumped in the root canal space with a sterile #15 K-file. Excess inoculum was wiped off the tooth with an alcohol saturated gauze. The box sponges on the bottom of the boxes were saturated with 50ml of sterile ionized water for humidity and minimization of root and bacteria dehydration. Boxes were placed in the anaerobic chamber (80%N2, 10% H2, 10% CO2) at 37°C for 36 hrs, when the infection challenge was repeated with an additional 1ml of inoculum, and the boxes were placed back in the anaerobic chamber for another 36 hrs.

Pilot study

Pilot study was conducted to insure the consistency of bacterial infection. The smallest file size (20.04) was tested with positive and negative controls (5 teeth in each group, experiment repeated twice; total n=30). Negative control groups remained negative throughout the experiment, and were omitted from future inclusions. Sampling occurred before instrumentation, immediately after instrumentation, 3, 7, 10 and 14 days after instrumentation. Before instrumentation, results showed 80% success rate of establishing infection with single infection challenge, thus in future experiments, the infection challenge was repeated twice. There was no bacterial recovery immediately after instrumentation in any group. As the sampling days progressed, so did the number of bacteria in each consecutive sampling. Thus, sampling before and immediately after instrumentation were omitted from future experiments.

Inhibitory effects of the irrigants (NaOCl, EDTA, sodium thiosulfate, TSB and phosphate buffered saline solution) on E. *faecalis* were evaluated using agar diffusion test on Mueller-Hinton agar plate (Spectrum Scientific, Philadelphia, PA). It showed that NaOCl had the largest diameter of zone of inhibition of bacterial growth, followed by EDTA which showed half the diameter of NaOCl. The rest of the irrigants had no effect on the bacterial growth.

In deciding which bacteria to use, we compared stock culture of E. *faecalis* (American Type Culture Collection CC18) to a clinical isolate (total n=30). CC18 was easily eradicated from the teeth compared to the clinical isolate. Therefore, clinical isolate was used in future experiments.

One round of the experiment (total n=30) experienced a humidity issue in the anaerobic chamber, causing dehydration and inhibition of bacterial growth. Therefore, the results of that round were excluded from the final results, and the humidity was adjusted for future experiments.

Experimental method

Sterilized boxes were randomly assigned to experimental or control groups. Brands and sizes used are listed in Table 1.1.

Group	Brand	Manufacturer	Size
Experimental			
A	TF Adaptive	SybronEndo, Orange, CA	20.04
B	ProTaper Gold	Dentsply Tulsa, Tulsa, OK	20.07
C	TF Adaptive	SybronEndo, Orange, CA	25.06
D	ProTaper Gold	Dentsply Tulsa, Tulsa, OK	25.08
E	Vortex	Dentsply Tulsa, Tulsa, OK	30.06
F	Typhoon	Clinician's Choice, New Milford, CT	35.06
Positive control			
A-PC	TF Adaptive	SybronEndo, Orange, CA	20.04
E-PC	Vortex	Dentsply Tulsa, Tulsa, OK	30.06
F-PC	Typhoon	Clinician's Choice, New Milford, CT	35.06

Table 1.1. Brands and sizes of NiTi rotary files used

The experiment was repeated three times (total n=75). Instrumentation protocol followed manufacturer's guidelines for each file system used. The irrigation protocol was as follows: Experimental Groups: 3ml 6%NaOCl (during instrumentation) + 1.5ml 17% EDTA + 3ml 6% NaOCl + 2ml 5% sodium thiosulfate solution + 1ml of 0.9% phosphate buffered saline solution

Positive Control Groups: 7.5ml phosphate buffered saline solution +2ml 5% sodium thiosulfate solution + 1ml of 0.9% phosphate buffered saline solution.

Sample collection occurred 3, 7, 10 and 14 days after instrumentation. Three paper points (Maillefer, Patterson Dental, St. Paul, MN) size "fine" were used per root. Paper points were transferred to vials containing liquid TSB with microbeads, vortexed for 30 seconds, and immediately plated on non-selective media agar plates (TSA 5% SB, Spectrum Scientific, Philadelphia, PA) using model D spiral plater. Plates were incubated aerobically for 24hrs at 37°C. Growth on the plates was counted using ProtoCOL automated colony counter and plate reader.

Teeth were replenished with 1ml of TSB every 36-48 hours (either immediately after sample collection, or in between sampling days) in order to allow E. *faecalis* that might have been shocked during the irrigation protocol to recover.

Statistical analysis

Outcome variable (bacterial count) was classified into three ordinal categories [<2.77 (no detectable growth), 2.78-4.99 (transitional growth), >5.0 (established infection)] and the bacterial count was evaluated twice (day 3 and 14). To evaluate for differences between six experimental groups over time, data was analyzed using proportional odds model for repeated measures with independent working correlation. In order to see if there was a difference between the experimental group and its respective control group, the change in bacterial count score from day 3 to day 14 was compared using the Mantel-Haenszel row mean score statistic with modified ridit scores. The significance level was set at 5% ($p < 0.05$).

RESULTS

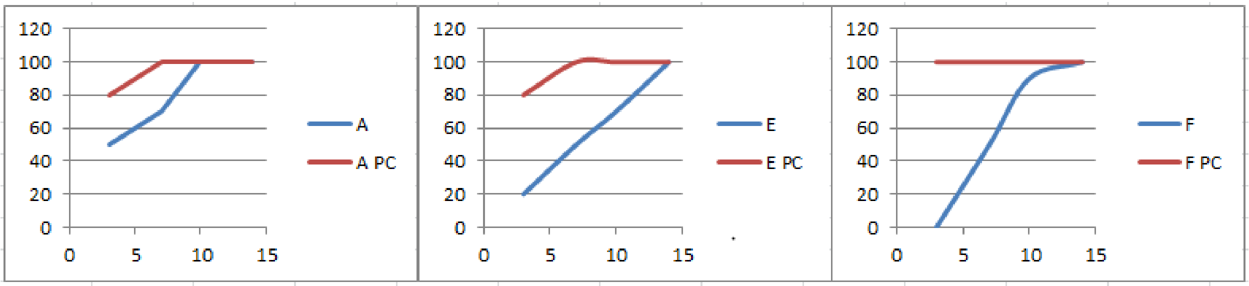
The pattern of change over time did not differ between the six experimental groups (p-value=0.0870). There was a significant overall group difference, averaged over time (i.e., at least two of the groups differed from each other significantly) (p-value= 0.0133) and there was a significant overall difference between the two time points (averaged over the six groups) (p-value <0.0001) with respect to the likelihood of higher bacteria count.

Using pairwise comparison of odds ratios (Table 1.2), it was found that group E (30.06) was significantly different from groups B (20.07), C (25.06) and D (25.08); group F (35.06) was also significantly different from groups B, C and D with respect to the likelihood of higher bacteria count. There was no difference between groups A (20.04), E (30.06) and F (35.06).

Groups	**OR**	**SE**	**95% CIs**		**p-value**
A vs. B	0.42	0.34	0.08	2.10	0.2891
A vs. C	0.39	0.28	0.09	1.62	0.1948
A vs. D	0.30	0.30	0.04	2.12	0.2248
A vs. E	1.99	1.61	0.40	9.77	0.3982
A vs. F	2.50	1.74	0.64	9.77	0.1865
B vs. C	0.93	0.61	0.26	3.35	0.9096
B vs. D	0.71	0.67	0.11	4.59	0.7161
B vs. E	4.76	3.64	1.06	21.35	0.0417
B vs. F	6.00	3.81	1.73	20.84	0.0048
C vs. D	0.76	0.66	0.14	4.17	0.7532
C vs. E	5.13	3.43	1.38	19.00	0.0145
C vs. F	6.46	3.33	2.35	17.75	0.0003
D vs. E	6.73	6.44	1.03	43.86	0.0460
D vs. F	8.49	7.24	1.59	45.16	0.0122
E vs. F	1.26	0.79	0.37	4.34	0.7138
Day: 3 vs. 14	0.02	0.01	0.01	0.05	0.0001

Table 1.2. Pairwise comparison of odds ratios. A 20.04, B 20.07, C 25.06, D 25.08, E 30.06, F 35.06

In order to see whether there was a difference between an experimental group and its respective control group, the change in bacterial count score from day 3 to day 14 was compared. The experimental group differed significantly from the control group for all 3 comparisons (Figure 1.2).



(1) (2) (3)

Figure 1.2. Percentage of bacterial infection between days 3 and 14 for experimental groups and their respective controls. (1) A 20.04, (2) E 30.06, (3) F 35.06.

DISCUSSION

In this study, we evaluated the differences in bacterial reduction between different sizes of rotary preparations while using a 31-gauge irrigation needle. A number of studies have been done evaluating the debris removal and bacterial reduction and elimination using irrigation needles ranging in size from 22- to 30-gauge (7, 8, 17, 26-28). They all concluded that the higher number gauge narrower diameter needle is better because it allows more apical placement within the root, thus delivering the irrigant to the most apical portion. This was why we decided to test the 31-gauge needle. According to the ISO 9626 tubing specification (29), size 31-gauge needle has a designated metric size of 0.25, with the outside diameter range of 0.254-0.267mm, and a minimal inside diameter of 0.114mm. Therefore, in a canal that has been prepared to an apical size of 30, a 31-gauge needle should be able to reach all the way to the apex. In testing of this hypothesis, we wanted to see if it would be possible to instrument the canals to a smaller size than is currently recommended (15) and achieve a desired goal.

Enterococcus faecalis is a Gram+ aerotolerant bacteria commonly found in the gastrointestinal tract. It is also frequently encountered in secondary endodontic infections (30-32). E. *faecalis* is known for its resilient nature and capability of surviving under extreme conditions and low nutritional availability. It is an exceptionally challenging bacteria to eradicate, which is why it was chosen for this experiment. Besides being in a planktonic state, E. *faecalis* has the ability to form a biofilm within the root (33), and it has been shown that microorganisms in the biofilm have different phenotypes then their planktonic counterparts (34). Dunavant *et al*. (35) showed when E. *faecalis* was put under stress, it induced a more resilient biofilm. Stress has also been shown to induce increased E. *faecalis* penetration into the dentinal tubules (33). All of these findings contribute to the difficulties in eradication attempts of E. *faecalis*.

The results of our study showed that smaller canal preparations harbored more bacteria over the tested time period compared to the larger sizes, however, the pattern of change over time was the same for all the groups. This is because E. *faecalis* grows exponentially, so as long as there is at least one colony of E. *faecalis* left after instrumentation and irrigation, if the conditions are favorable, it will rebound.

Groups 20.04, 20.07, 25.06 and 25.08 showed no difference between each other when evaluating for likelihood of higher vs. lower bacterial count controlling for days (day 3 and day 14). The same was found between groups 30.06 and 35.06. There was a statistically significant difference between these two groups and all the smaller sizes groups except for 20.04 group. This could be explained by the fact that with the size 20.04, the 31-gauge needle reached within three millimeter from the apex. Therefore, the TSB replenishment may not have been adequate, and it took longer for the bacteria to rebound after the initial shock of instrumentation and irrigation. Therefore, our bacterial recovery during sampling may not have been as successful. For the purpose of comparing different preparation sizes and the amount of bacterial reduction, we decided to consider the 20.04 group an outlier.

Controlling for group, the likelihood of higher bacteria count (vs. lower bacteria) at day 3 was 0.02 times less likely than that at day 14 (95% CI: 0.01–0.05), which was statistically significant.

When comparing the experimental groups to their matching controls, the results showed significant difference between the two. Positive control groups showed positive bacterial growth early on, but the experimental groups caught up over time. This was another proof that the bacteria are not eliminated during instrumentation and irrigation regardless of the type of irrigant used, and if the conditions for the bacterial growth are favorable, they will regain their ground and proliferate.

In conclusion, within the limitations of this study, our results support those by Khademi *et al.* (15) in stating that 30.06 was the minimal size of root canal preparation needed to notice a statistically significant difference in bacterial reduction when compared to smaller sizes during the testing period. The use of a 31-gauge irrigation needle did not show an increased effect on bacterial elimination in smaller preparation sizes. Since E. *faecalis* cannot be eradicated from the root canal system, future research could evaluate the same sizes and 31-gauge needle without the bacterial infection (similar to Khademi's 2006 study) (15), and evaluate the amount of debris and smear layer removal in the apical portion of the root under the SEM.

REFERENCES

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The Effects of Surgical Exposures of Dental Pulps in Germ-Free and Conventional Laboratory Rats. Oral surgery, oral medicine, and oral pathology 1965;20:340-349.

2. Moller AJ, Fabricius L, Dahlen G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scandinavian journal of dental research 1981;89(6):475-484.

3. Peters OA, Peters CI. Cleaning and shaping of the root canal system. In: Cohen S, Pathways of the Pulp. 10th edition, St. Louis, MO: Mosby Company, 2011: 283-348.

4. Sundqvist G, Figdor D. Endodontic treatment of apical periodontitis. In: Orstavik D, Pitt Ford TR, eds. Essential Endodontology: Prevention and Treatment of Apical Periodontitis. London, UK. Blackwell Science Ltd. 1998:242-269.

5. Peters OA, Schonenberger K, Laib A. Effects of four Ni-Ti preparation techniques on root canal geometry assessed by micro computed tomography. International endodontic journal 2001;34(3):221-230.

6. Baker NA, Eleazer PD, Averbach RE, Seltzer S. Scanning electron microscopic study of the efficacy of various irrigating solutions. Journal of endodontics 1975;1(4):127-135.

7. Siqueira JF, Jr., Rocas IN, Favieri A, Lima KC. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. Journal of endodontics 2000;26(6):331-334.

8. Card SJ, Sigurdsson A, Orstavik D, Trope M. The effectiveness of increased apical enlargement in reducing intracanal bacteria. Journal of endodontics 2002;28(11):779-783.

9. Peters LB, Wesselink PR. Periapical healing of endodontically treated teeth in one and two visits obturated in the presence or absence of detectable microorganisms. International endodontic journal 2002;35(8):660-667.

10. Fujii R, Saito Y, Tokura Y, Nakagawa KI, Okuda K, Ishihara K. Characterization of bacterial flora in persistent apical periodontitis lesions. Oral microbiology and immunology 2009;24(6):502-505.

11. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Critical reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists 2004;15(6):348-381.

12. Shemesh H, Bier CA, Wu MK, Tanomaru-Filho M, Wesselink PR. The effects of canal preparation and filling on the incidence of dentinal defects. International endodontic journal 2009;42(3):208-213.

13. Liu R, Kaiwar A, Shemesh H, Wesselink PR, Hou B, Wu MK. Incidence of apical root cracks and apical dentinal detachments after canal preparation with hand and rotary files at different instrumentation lengths. Journal of endodontics 2013;39(1):129-132.

14. Tawil PZ, Saraiya VM, Galicia JC, Duggan DJ. Periapical microsurgery: the effect of root dentinal defects on short- and long-term outcome. Journal of endodontics 2015;41(1):22-27.

15. Khademi A, Yazdizadeh M, Feizianfard M. Determination of the minimum instrumentation size for penetration of irrigants to the apical third of root canal systems. Journal of endodontics 2006;32(5):417-420.

16. De Deus QD. Frequency, location, and direction of the lateral, secondary, and accessory canals. Journal of endodontics 1975;1(11):361-366.

17. Guerreiro-Tanomaru JM, Loiola LE, Morgental RD, Leonardo Rde T, Tanomaru-Filho M. Efficacy of four irrigation needles in cleaning the apical third of root canals. Brazilian dental journal 2013;24(1):21-24.

18. Hockett JL, Dommisch JK, Johnson JD, Cohenca N. Antimicrobial efficacy of two irrigation techniques in tapered and nontapered canal preparations: an in vitro study. Journal of endodontics 2008;34(11):1374-1377.

19. Albrecht LJ, Baumgartner JC, Marshall JG. Evaluation of apical debris removal using various sizes and tapers of ProFile GT files. Journal of endodontics 2004;30(6):425-428.

20. Abou-Rass M, Piccinino MV. The effectiveness of four clinical irrigation methods on the removal of root canal debris. Oral surgery, oral medicine, and oral pathology 1982;54(3):323-328.

21. Chow TW. Mechanical effectiveness of root canal irrigation. Journal of endodontics 1983;9(11):475-479.

22. Sedgley CM, Nagel AC, Hall D, Applegate B. Influence of irrigant needle depth in removing bioluminescent bacteria inoculated into instrumented root canals using real-time imaging in vitro. International endodontic journal 2005;38(2):97-104.

23. Baratto-Filho F, Leonardi DP, Zielak JC, Vanni JR, Sayao-Maia SM, Sousa-Neto MD. Influence of ProTaper finishing files and sodium hypochlorite on cleaning and shaping of mandibuldar central incisors--a histological analysis. Journal of applied oral science : revista FOB 2009;17(3):229-233.

24. Coldero LG, McHugh S, MacKenzie D, Saunders WP. Reduction in intracanal bacteria during root canal preparation with and without apical enlargement. International endodontic journal 2002;35(5):437-446.

25. Falk KW, Sedgley CM. The influence of preparation size on the mechanical efficacy of root canal irrigation in vitro. Journal of endodontics 2005;31(10):742-745.

26. Siqueira JF, Jr., Rocas IN, Santos SR, Lima KC, Magalhaes FA, de Uzeda M. Efficacy of instrumentation techniques and irrigation regimens in reducing the bacterial population within root canals. Journal of endodontics 2002;28(3):181-184.

27. Cohenca N, Paranjpe A, Heilborn C, Johnson JD. Antimicrobial efficacy of two irrigation techniques in tapered and non-tapered canal preparations. A randomized controlled clinical trial. Quintessence international 2013;44(3):217-228.

28. Huang TY, Gulabivala K, Ng YL. A bio-molecular film ex-vivo model to evaluate the influence of canal dimensions and irrigation variables on the efficacy of irrigation. International endodontic journal 2008;41(1):60-71.

29. International Standards Organization. Stainless steel needle tubing for the manufacture of medical devices. ISO 9626:1991/Amd.1:2001(E)

30. Molander A, Reit C, Dahlen G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. International endodontic journal 1998;31(1):1-7.

31. Peciuliene V, Maneliene R, Balcikonyte E, Drukteinis S, Rutkunas V. Microorganisms in root canal infections: a review. Stomatologija / issued by public institution "Odontologijos studija" ... [et al.] 2008;10(1):4-9.

32. Rocas IN, Siqueira JF, Jr., Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. Journal of endodontics 2004;30(5):315-320.

33. George S, Kishen A, Song KP. The role of environmental changes on monospecies biofilm formation on root canal wall by Enterococcus faecalis. Journal of endodontics 2005;31(12):867-872.

34. Li YH, Chen YY, Burne RA. Regulation of urease gene expression by Streptococcus salivarius growing in biofilms. Environmental microbiology 2000;2(2):169-177.

35. Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against Enterococcus faecalis biofilms. Journal of endodontics 2006;32(6):527-531.

CHAPTER 3 MANUSCRIPT #2
NOMINAL SIZE AND TAPER ANALYSIS OF NOVEL METALLURGY NiTi FILES

INTRODUCTION

Manufacturers of endodontic files rely on the specifications set by the International Standards Organization (ISO 3630-1) (1) and American National Standards Institute/American Dental Association (ANSI/ADA Spec 101) (2) to ensure their files specifications fall within the allowable tolerance for their advertised sizes. These guidelines exist thanks to the pioneering efforts of Green (3), Ingle (4, 5), and others who called for standardization of stainless steel 02 taper endodontic instruments and obturation materials in the mid-1950's. Current guidelines for nickel-titanium (NiTi) rotary files of any size set the diameter tolerance to be within 50% of the difference between the next smaller and/or larger instrument size (2), while the allowable taper tolerance is set to be within 0.05 of the advertised taper size (2). In other words, for the diameter, if the file's size increases by 0.05mm, then the allowable tolerance would be ±0.025mm from the advertised size. For the taper, the generous allowance of ±0.05 means that if the file is advertised as having a 06 taper, the actual file taper can be anywhere from 01 taper to 11 taper, and still satisfy the ANSI/ADA guideline.

In 2002, Zinelis *et al*. (6) reported on the clinical relevance of standardization rules of ISO 3630-1 specification, and concluded that due to the large amount of allowable tolerance, even though the tested files were not the size they were advertised to be, they still fell within the acceptable tolerance range. A number of studies looked at the continuous taper NiTi rotary files diameters and tapers (6-11), but to our knowledge, there has not been a publication comparing dimensional values of conventional NiTi rotary files with the newer heat-treated NiTi files. When a file is heat-treated, the flexibility of the file is increased, which may lead to a greater affinity for distortion during use (12). If this happens, then the prepared canal size may not be the size of the file that was used, and it may pose a disinfection challenge and unnecessary frustration to the practitioner when choosing a proper size gutta percha for obturation (13).

The ISO and ANSI/ADA publications did not set a guideline for the file's pilot tip length; they left it up to manufacturer's discretion. Pilot tip (Figure 2.1) is the area of the file from the very tip of the file to the first cutting edge. Its purpose is to enlarge the canal and to help guide the file through it (14). The distance from the tip of the file to where the advertised size of the file is actually located, is what we termed a true pilot tip length. To our knowledge, there have been no reports on evaluation of the true pilot tip length, and we hope to bridge that gap in knowledge.

The purpose of this study was to evaluate the nominal tip diameter, taper and true pilot tip length of NiTi rotary files before and after use, and to evaluate manufacturer's compliance with the ANSI/ADA guidelines as well as our stricter, new recommended guideline (NRG).

MATERIALS AND METHODS

Five different brands of files were used; three in size 25.08: ProTaper Universal (Dentsply, Tulsa Dental, Tulsa, OK), ProTaper Gold (Dentsply, Tulsa Dental, Tulsa, OK), and Channels Progressive Taper (Insight Endo for Henry Schein, Melville, NY), and two in size 35.06: Vortex Blue (Dentsply, Tulsa Dental, Tulsa, OK) and Typhoon Infinite Flex NiTi files (Clinician's Choice, Dental Products Inc, New Milford, CT). Ten files were used from each brand (total n=50). Prior to measurements, files were steam sterilized, conditioned for at least ten hours at 20$\pm$5° C, and imaged under SEM microscope (FEI Quanta 200F Environmental

Scanning Electron Microscope (ESEM), FEI Inc. Hillsboro, OR) at 50x magnification and a resolution of 0.1 micron.

All images were evaluated and measured by two independent and equilibrated examiners using ImageJ software (National Institute of Health, Bethesda, MD). The agreement was set at 0.001mm.

Nominal diameter (D0) was measured from the cutting tip of the file (Figure 2.1). If the obtained value did not correspond to the advertised size of the file, a measurement was made down the length of the file until the measurement of the advertised size was reached. This was termed "True D0". This value was used for true pilot tip length determination (from the tip of the file to the "True D0").

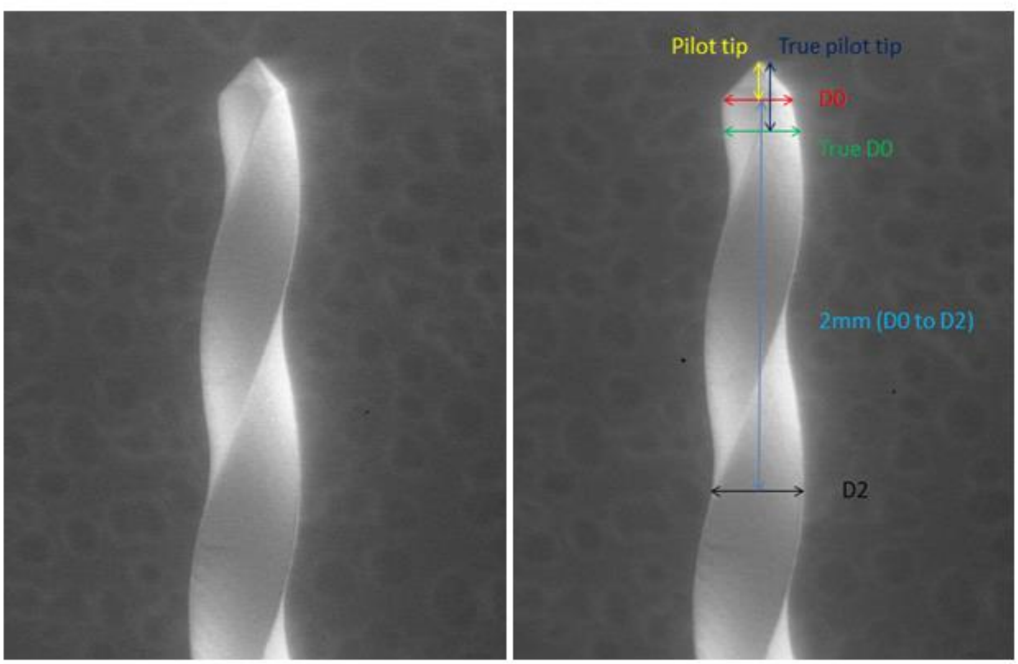


Figure 2.1. SEM image with measurement schematics. **D0** – distance from cutting edge across the file. **True D0** – location of the advertised file size. **D2** – diameter 2mm from D0. **Pilot tip** – distance from the tip of the file to D0. **True pilot tip** – distance from the tip of the file to True D0.

Taper of the file was measured using the following formula set by ANSI/ADA Spec 101:

$$\text{Taper} = \frac{\text{Distance of diameters}}{\text{Distance between diameters}}$$

The guideline states that the diameters included in the above equation are D0 and D16 (or alternatively D3 and D16). We made a protocol adjustment here due to the variable taper of the ProTaper and Channels files beyond the initial 4mm of the file length. Therefore, the diameters included in our analysis were D0 and D2. Differences between the measured taper and the advertised taper were determined.

Pilot tip length was measured from the tip of the file to D0. True pilot tip length was measured from the tip of the file to where the advertised size of the file was recorded (True D0).

After initial measurements, instrumentation was performed on plastic endodontic training blocks (Brasseler, Savannah, GA) according to each file's manufacturer's recommendation. After instrumentation, files were conditioned and imaged under the SEM again. The same measurements were repeated as in the initial set up.

Data analysis was conducted using repeated measures analysis of variance (r-ANOVA) and Tukey's test to evaluate the differences between the diameter, taper and true pilot tip length before and after use and compared to their advertised sizes. The P-value was set at <0.05. An observation was made of the percentages of files that satisfied the ANSI/ADA tolerance level ($\pm$0.025mm for diameter, and $\pm$0.05 for the taper), and our new recommended guideline (NRG) ($\pm$0.0125mm for the diameter, and $\pm$0.005 for the taper).

RESULTS

The mean diameter values are recorded in Table 2.1. None of the files had the advertised nominal diameter size. The mean measure of D0 in each group was significantly different from the advertised size; the mean D0 measure was less than the advertised size for all brands except for the Channels files. There was no significant mean difference between the before and after measures of D0 in each group except for the ProTaper Universal group.

	File Brand	Before		After		Deformation Before vs. After	
		Mean D0	p-value	Mean D0	p-value	MEAN	p-value
	ProTaper Universal	0.231±0.007	<.0001	0.225±0.006	<.0001	+0.006±0.007	0.0278
25.08	Channels	0.276±0.016	0.0005	0.272±0.008	<.0001	+0.004±0.005	0.3704
	ProTaper Gold	0.219±0.016	0.0002	0.211±0.011	<.0001	+0.007±0.017	0.1815
35.06	Vortex Blue	0.244+0.017	<.0001	0.243+0.025	<.0001	+0.001±0.013	0.7483
	Typhoon	0.311+0.015	<.0001	0.305+0.020	<.0001	+0.006±0.018	0.3158

Table 2.1. Diameter values (before and after) compared to the advertised nominal size.

Table 2.2 shows the percentage of the files that were within the allowable tolerance set by ANSI/ADA Spec 101, as well as our new recommended tolerance limits (NRG). ProTaper Universal group had the most files that satisfied the ANSI/ADA tolerance limit. All other files had a tendency to be smaller than the allowable range EXCEPT for the Channels files, which tended to have larger diameter than the allowable ANSI/ADA range.

	File Brand	**Before**		**After**	
		ANSI/ADA 0.225-0.275	**NRG** 0.2375-0.2625	**ANSI/ADA** 0.225-0.275	**NRG** 0.2375-0.2625
25.08	ProTaper Universal	80% (20,0)	0% (60,40)	60% (40,0)	0% (80,20)
	Channels	50% (0,50)	30% (10,60)	60% (0,40)	10% (0,90)
	ProTaper Gold	20% (80,0)	10% (90,0)	10% (90,0)	0% (100,0)
		ANSI/ADA 0.325-0.375	**NRG** 0.3375-0.3625	**ANSI/ADA** 0.325-0.375	**NRG** 0.3375-0.3625
35.06	Vortex Blue	0% (100,0)	0% (100,0)	0% (100,0)	0% (100,0)
	Typhoon	20% (80,0)	0% (100,0)	30% (70,0)	10% (90,0)

Table 2.2. Percentage of files within the ANSI/ADA and NRG (new recommended guideline) diameter tolerance limit. Numbers in parenthesis show percentage of files below and above the tolerance limit, respectively.

Taper values indicated that the mean measure of taper in each group was significantly different from the advertised taper both before and after use except for the ProTaper Gold group. In the ProTaper Universal, Channels and the Typhoon groups, the mean taper measure (before and after) were less than the advertised size; whereas in the ProTaper Gold and the Vortex Blue groups, the mean taper measure (before and after) was greater than the advertised size. These findings are summarized in Table 2.3. There was no statistically significant mean change (before vs. after) in any group.

	File Brand	**Before**		**After**		**Deformation before vs. after**	
		Mean Taper	**p-value**	**Mean Taper**	**p-value**	**MEAN**	**p-value**
25.08	ProTaper Universal	0.073±0.003	<.0001	0.073±0.002	<.0001	+0.000±0.003	0.6417
	Channels	0.056±0.007	<.0001	0.058±0.007	<.0001	-0.002±0.007	0.6828
	ProTaper Gold	0.085±0.008	0.105	0.088±0.009	0.0234	-0.003±0.009	0.3510
35.06	Vortex Blue	0.074±0.009	0.0007	0.077±0.007	<.0001	-0.003±0.007	0.1722
	Typhoon	0.049±0.006	0.0003	0.053±0.007	0.0202	-0.004±0.010	0.1978

Table 2.3. Taper values (before and after) compared to the advertised nominal taper sizes.

Table 2.4 shows the percentages of files within each tolerance level (ISO and NRG). All the files in all groups were within the allowable ISO taper tolerance. 40% of ProTaper Universal files, 20% of ProTaper Gold files, 20% of Vortex Blue files and 30% of Typhoon files were the exact taper as advertised. None of the Channels files had the advertised nominal taper size.

When compared to the NRG range, ProTaper Universal, ProTaper Gold, Vortex Blue and Typhoon files were within the allowable range 30% of the time. 100% of Channels files were smaller than NRG range. Findings are summarized in the table below (Table 2.4).

	File Brand	**Before**		**After**	
		ANSI/ADA 0.03-0.13	**NRG** 0.075-0.085	**ANSI/ADA** 0.03-0.13	**NRG** 0.075-0.085
25.08	ProTaper Universal	100% (0,0)	30% (70,0)	100% (0,0)	20% (80,0)
	Channels	100% (0,0)	0% (100,0)	100% (0,0)	0% (100,0)
	ProTaper Gold	100% (0,0)	30% (10,60)	100% (0,0)	40% (10,50)
		ANSI/ADA 0.01-0.11	**NRG** 0.055-0.065	**ANSI/ADA** 0.01-0.11	**NRG** 0.055-0.065
35.06	Vortex Blue	100% (0,0)	30% (0,70)	100% (0,0)	10% (0,90)
	Typhoon	100% (0,0)	30% (70,0)	100% (0,0)	40% (60,0)

Table 2.4. Percentage of files within the ANSI/ADA and NRG taper tolerance limit. Numbers in parenthesis show percentage of files below and above the tolerance limit, respectively.

The mean true pilot tip lengths varied substantially between different rotary systems (Table 2.5), from 0.21mm to 1.45mm. There was no significant mean change (before and after measures) of the true pilot tip length for any group except for the Typhoon group, where the mean true pilot tip length before use (0.883mm) was significantly different from the mean length after use measurement (1.030mm). Findings are summarized in the table below (Table 2.5).

	File Brand	Before	After	Before vs. After	
		Mean TPT	Mean TPT	MEAN	p-value
25.08	ProTaper Universal	0.41±0.10	0.40±0.06	+0.01±0.09	0.8287
	Channels	0.22±0.01	0.21±0.01	-0.00±0.020	0.9542
	ProTaper Gold	0.40±0.07	0.41±0.05	-0.01±0.080	0.6163
35.06	Vortex Blue	1.45±0.16	1.42±0.20	+0.03±0.123	0.4176
	Typhoon	0.88±0.15	1.03±0.11	-0.15±0.103	0.0015

Table 2.5. True pilot tip (TPT) lengths (before and after)

DISCUSSION

The latest revisions and reaffirmations to the ISO 3630-1 and ANSI/ADA Spec 101 were done in 2008 and 2010, respectively (1, 2). Even though the guidelines are available, there are still variations in dimensional standards being reported (6-10). These guidelines were what we used in our evaluation of the nominal diameter and taper analysis of three brands of size 25.08 and two brands of 35.06 files, as well as our new stricter recommended guideline (NRG). We also evaluated the lengths of the pilot tips of all the files.

Evaluation of stainless steel H- and K-files, as well as rotary NiTi files by Zinelis *et al*. (6) showed that none of the files had the advertised nominal diameter. Lask *et al*. (10) evaluated diameter and taper of four different brands of size 30.04 NiTi rotary files, and found that files tended to be larger than their nominal sizes, but concluded that the differences were minute and probably not clinically relevant. Kim *et al*. (9) evaluated dimensional standard of several NiTi rotary files, and found that the diameter values were mostly not in compliance with the advertised values. In our current investigation, none of the files tested had the advertised nominal tip diameter ($p<0.05$). For the 25.08 size, the range was from 21mm to 28mm. ProTaper Universal and ProTaper Gold files tended to be smaller than the nominal size, and the Channels files tended to be larger than the advertised nominal size. A statistically significant difference was noted in the ProTaper Universal group between before and after measurement, however, with the mean diameter difference of 0.006mm and a standard deviation of $\pm$0.007mm, this deformation would probably not be clinically relevant.

For the 35.06 size, both groups displayed smaller nominal diameter sizes than advertised, ranging from 24mm for the Vortex Blue group, to 31mm for the Typhoon group.

The percent of files that were within the ANSI/ADA tolerance limit was 80% for the ProTaper Universal, 50% for the Channels, 20% for both the ProTaper Gold and the Typhoon groups, and none for the Vortex Blue group. There was a tendency for the files to be on the smaller size for all of the groups except for the Channels group, where a consistently larger than the highest ANSI/ADA limit was observed. The same trend was noted within our new tolerance limit as well, with the smaller percentage of files satisfying the tolerance range. It is interesting to observe that the austenite phase files (ProTaper Universal and Channels) had more files that were within the ANSI/ADA tolerance limit compared to the newer metallurgy heat-treated (ProTaper Gold and Vortex Blue) and martensite phase files (Typhoon). It is also of interest that even though files may be advertised as the exact replicas of one another (ProTaper Universal and Channels), practitioner should be aware of a potential size difference, especially if using a hybrid technique with rotary instrumentation. The mean nominal size of ProTaper Universal was 23mm, and for the Channels was 28mm. Zinelis *et al*. (6) pointed out the issue of file size overlap, where the next bigger size may be the same as the last smaller size. Practitioner should be aware of this possibility in order to minimize potential iatrogenic mishap during endodontic treatment. It should also be pointed out that if the file is not the size that it is advertised to be, the shaping of the canal space may not be adequate and it may prevent full irrigant delivery to the root canal system.

With the objective of endodontic therapy being thorough cleaning and shaping of the root canal system followed with an apical seal, it is important to have the obturation material of the same size as the prepared canal space, i.e. as the last instrument used in the canal preparation. Cunningham *et al*. (13) found significant variability between different brands of gutta percha size 30.04 in both diameter and taper, and none of the brands tested had the nominal advertised size. Practitioner should be aware that not only files may be of different sizes than advertised, but the matching gutta percha may not be a match at all. Therefore, a stricter adherence by the manufacturers to the set guidelines should be recommended.

Taper evaluation indicated that only ProTaper Gold had the mean taper that was not statistically different from the advertised taper. However, all the files satisfied the ANSI/ADA tolerance range. This is in agreement with previous reports (7, 8). In the 25.08 group, mean taper for the ProTaper Universal was 07 and for the Channels was 06. For the 35.06 group, the mean taper for the Vortex Blue was 07 and for the Typhoon group was 05.

When evaluating compliance with our new recommended guidelines (NRG), 30% agreement was noted for all the files except for the Channels group. When not in compliance, ProTaper Universal, Channels and Typhoon files were generally smaller than the set tolerance range, and ProTaper Gold and Vortex Blue files were generally larger. Again a pattern was observed, where the austenite phase files acted in the similar fashion (ProTaper Universal and Channels), as did the heat-treated files (ProTaper Gold and Vortex Blue). Further investigation might be warranted. It should also be pointed out that the current ANSI/ADA Spec 101 guidelines for taper tolerance of $\pm$0.05 are rather generous, and a stricter updated guideline should be recommended.

Evaluation of the true pilot tip length pointed to the complete lack of standardization between different files and manufacturers. The range in true pilot tip length was truly extraordinary, from 0.21mm for the Channels group to 1.45mm for the Vortex Blue group. ProTaper Universal and ProTaper Gold displayed almost identical true pilot tip lengths of 0.41mm and 0.40mm, respectively. Typhoon files were the only ones that displayed a significant difference in the before vs. after measurement (0.883mm to 1.030mm). This is in agreement with Peters' (12) report of martensite file deformation under stress. Such large variations in true pilot tip lengths might be of clinical importance and warrants further research.

In summary, the present study showed that the nominal diameter sizes of the files tested and most of their taper values are not in agreement with their advertised sizes. Clinical relevance might be argued, but stricter expectations from the manufacturers should be recommended in order to minimize potential for sizing overlap, practitioner frustration and iatrogenic complications. We would also like to propose that a guideline should be set to standardize the length of the pilot tip. Future studies should look at other sizes and brands of files and evaluate for differences between different metallurgies.

REFERENCES

1. International Standards Organization. Dentistry - Root-canal instruments. Part 1: General Requirements and Test Methods. ISO 3630-1:2008(E). 2008.

2. American Dental Association Council on Scientific Affairs. ANSI/ADA Specification No. 101. Root Canal Instruments: General Requirements. 2001. Reaffirmed Oct 2010.

3. Green EN. Microscopic investigation of root canal file and reamer widths. Oral surgery, oral medicine, and oral pathology. 1957;10(5):532-40.

4. Ingle JI. The need for endodontic instrument standardization. Oral surgery, oral medicine, and oral pathology. 1955;8(11):1211-3.

5. Ingle JI. A standardized endodontic technique utilizing newly designed instruments and filling materials. Oral surgery, oral medicine, and oral pathology. 1961;14:83-91.

6. Zinelis S, Magnissalis EA, Margelos J, Lambrianidis T. Clinical relevance of standardization of endodontic files dimensions according to the ISO 3630-1 specification. Journal of endodontics. 2002;28(5):367-70.

7. Gergi R, Abou Rjeily J, Osta N, Sader J, Naaman A. Taper preparation variability compared to current taper standards using computed tomography. International journal of dentistry. 2012;2012:265695.

8. Hatch GW, Roberts S, Joyce AP, Runner R, McPherson JC, 3rd. Comparative study of the variability of 0.06 tapered rotary endodontic files to current taper standards. Journal of endodontics. 2008;34(4):463-5.

9. Kim KW, Cho KM, Park SH, Choi KY, Karabucak B, Kim JW. A comparison of dimensional standard of several nickel-titanium rotary files. Restorative dentistry & endodontics. 2014;39(1):7-11.

10. Lask JT, Walker MP, Kulild JC, Cunningham KP, Shull PA. Variability of the diameter and taper of size #30, 0.04 nickel-titanium rotary files. Journal of endodontics. 2006;32(12):1171-3.

11. Stenman E, Spangberg LS. Root canal instruments are poorly standardized. Journal of endodontics. 1993;19(7):327-34.

12. Peters OA, Gluskin AK, Weiss RA, Han JT. An in vitro assessment of the physical properties of novel Hyflex nickel-titanium rotary instruments. International endodontic journal. 2012;45(11):1027-34.

13. Cunningham KP, Walker MP, Kulild JC, Lask JT. Variability of the diameter and taper of size #30, 0.04 gutta-percha cones. Journal of endodontics. 2006;32(11):1081-4. Epub 2006/10/24.

14. McSpadden JT. Mastering Endodontic Instrumentation (1st edition). Chattanooga, TN: Cloudland Institute. 2007.